

# COMPARATIVE HISTOLOGY OF THE FEMALE INFLORESCENCE OF *ZEA MAYS* L.<sup>1</sup>

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## INTRODUCTION

While an interest in the corn cob has been shown by those who have attempted to explain the origin and nature of the ear, the increasing role which the cob is assuming today in industry, in archaeology, and in studies of the origin of maize makes it imperative that a careful comparative study of the cob be undertaken.

For many years corn cobs played no special role in the economy of corn production since approximately 90 per cent of the corn produced in the United States was used on the farms for feeding of livestock and most of it was fed as unshelled ears. The cobs remaining from ears that were shelled for poultry feed and the like were often used for fuel. However, with the advent of hybrid corn and large seed-processing plants, cobs have become a major by-product of a rapidly expanding agricultural industry and today ways and means are being sought for their utilization. Of the various uses suggested, one of the most promising is that of a filler in commercially processed feeds. There is some experimental evidence on the feeding value of ground cobs (Gerlaugh and Rogers, 1936), and further experiments are now under way. Other possibilities include its use in the production of furfural or as a substitute for peat-moss.

The corn cob plays an even more important role in the hybrid seed-corn industry. Since the grains of corn are produced on the cob, the physical characteristics of that structure affect the handling of the ear during processing. It is known, for instance, that certain corns are more difficult to shell than others, some shatter easily, and in some the grains are removed with difficulty. Some cobs are soft while others are hard and tough; certain cobs are very susceptible to ear-rotting fungi, while others are rarely attacked by these organisms. Since these differences are of considerable importance to the practical corn-breeder it is necessary that the anatomical, histological, and chemical reasons for these differences be understood.

There is another reason why a comparative study of the maize cob is desirable. Both in North and South America archaeologists have excavated a large number of prehistoric sites and have found maize remains in many of these excavations. A few of the best finds have yielded remarkably well-preserved ears, without even a kernel missing, although the ear may have been stored for many centuries. However, at least 90 per cent of the material in archaeological collections is cobs. Since the mature cob is a hard and durable structure and not particularly attrac-

<sup>1</sup>Part of an investigation carried out in the graduate laboratory of the Henry Shaw School of Botany of Washington University and submitted as a thesis in partial fulfilment of the requirements for the degree of Doctor of Philosophy. The investigation was made possible by a special fellowship from the Pioneer Hi-Bred Corn Co., of Des Moines, Iowa.

tive to insects and rodents, it will remain relatively unchanged in trash heaps for long periods of time if kept dry, and many of the richest finds have been made in extremely dry areas. In more humid areas often the only plant material found consists of carbonized cobs and in such areas the cob assumes an even more important role than it does in dry areas. Through a study of the surface features of the prehistoric cobs certain facts about the corn can be arrived at, as, for example, the general shape and size of the ear. However, an entirely new set of characters which are present in the cob must be utilized if we are to make the greatest use of the vast quantities of material collected by the archaeologists. Before we can do this it is necessary to know which characters vary and the extent of their variation, and this can only be determined by making careful comparative studies of the anatomical and histological features of the cobs of various kinds of maize.

There is a fourth reason why a comparative study of the female inflorescences of maize is needed, and that is for a study of the origin of maize. One of the best approaches to a study of the phylogeny of most groups of plants is through comparison of the reproductive structures of that group. This is especially true in the Gramineae where the vegetative structures are of less importance than in certain other families. Today there is as great a mass of literature concerning the origin of maize as for any other crop plant, yet there has never been a thorough comparative study of the anatomical and histological features of its female inflorescence. Before a basic understanding of maize can be reached such a study must be made, not only of maize but also of its close relatives. The present investigation is a beginning along such lines and is an attempt to determine which are the variable features in the cob and the extent of their variability in different kinds of maize.

#### REVIEW OF LITERATURE

The origin and nature of the ear of *Zea Mays* L. have been subjects for speculation since Indian corn was first described and many hypotheses have been presented to account for it. Finan (1948) has made a critical survey of the descriptions of corn as found in the literature of the early Spanish explorations and in the great herbals. Because the ear of maize was a structure different from anything with which they were familiar the early herbalists devoted considerable attention to that structure and apparently the earliest-known European illustration of the plant is a detailed drawing of a mature ear.

Although the ear of maize has been known and discussed in literature over such a long period there is no part of the corn plant about which there is such a paucity of anatomical information as the mature cob. On the whole, the investigators who have written most about the ear, aside from those describing varieties of corn, have been primarily concerned with its origin and with the homologies between the ear and the tassel. The literature on the subject has become voluminous. The reader is referred to Mangelsdorf's (1945) recent paper in which he discusses (pp. 33-73) the major hypotheses concerning the origin of the ear.

The principal reason for the lack of information about the anatomy of the cob is that it is a difficult structure to study in its mature state. A cursory examination of the cross-section of an ordinary corn cob discloses that it consists of very different types of tissue. The center of the cob contains large soft-pith parenchyma cells surrounded by a layer of stone cells of varying thickness which are often compacted to form tissue as hard as that found in the shell of a hickory nut. Interspersed in these two such different tissues is the tough-branched network of the vascular system. Weatherwax (1935) summed up the difficulties of studying the cob when he said:

"Long before maturity, however, the development of a tough sclerenchyma in the peripheral vascular region of the rachis makes it difficult to secure instructive sections even when the technique applicable to woody tissues is employed."

Not only is the cob hard and tough but it is composed of hard and soft tissues together, and if it is held firmly enough to make it possible to cut the hard parts the soft parts are apt to be crushed or broken.

While the anatomical features of the cob have not been well understood, the gross morphological features of the ear have been discussed by numerous investigators, especially in so far as those features could be used in describing corn varieties. John Lorain (1825) in his 'Nature and Reason Harmonized in the practice of Husbandry' described the varieties of corn grown in eastern United States in his time. It is evident from his book that he had a rather clear understanding not only of the basic types of corn grown in that region but even the effects brought about by the mixing of the different varieties. Bonafous, in 1836, monographed *Zea Mays*, describing the varieties of corn known to him, and in 1866 Enfield described a number of varieties. Sturtevant (1899) published the most comprehensive work on the classification of corn varieties that had appeared up to that time and which with only minor changes has been used until recently. Anderson and his collaborators (1942, 1946) have shown the need for a new classification of the races of corn which would incorporate all evidence available, especially the evidence from archaeology and genetics. Such a classification must be a goal toward which to work.

Most of the early authors, in their descriptions of corn, mention the cob and often remark about its strange character as, for example, Vasey (1884), when he wrote, "female spikes (originally by monstrous or teratological development?) are grown together into a spongy, continuous club-shaped body, the 'cob'." None of the early workers made comparative studies of the surface features of the cob. As Mangelsdorf (1945) has written, "though not easily interpreted [the ear] is not difficult to describe," and he describes it as "a spike upon whose thickened axis (the cob) naked grains (caryopses) are borne in longitudinal or somewhat spiral rows: eight, ten, twelve or more in number." Weatherwax (1935) gives a somewhat similar description, writing of it as "being made up of a cylindrical 'cob' around which the grains are arranged in eight or more parallel rows."

Collins (1919) is one of the first to have investigated the morphology of the ear in some detail. He brought in a new kind of evidence for this study by working with *Zea-Euchlaena* hybrids. He considered the primary morphological unit of the ear, as well as of the tassel, to be the organs borne in a single metamer of the rachis. In the pistillate inflorescence the members of this morphological unit occupy a single alveolus but since in the tassel the depression is too shallow to be termed an alveolus he proposed the word "alicole" to "designate the spikelet or spikelets, whether staminate or pistillate, that are borne in a single alveolus or at a single point on the rachis, considered as the axil or point of attachment of a reduced branch." According to Collins, there is evidence that the association of alicoles into pairs, "yoked alicoles," is more fundamental than their linear arrangement. In discussing dropping out of rows on the ear, Collins concluded that the dropped rows are on the opposite side of the ear and that this could occur if the two pediceled spikelets were dropped simultaneously from a pair of yoked alicoles. Weatherwax (1935) has shown that the rows of grains on the cob are paired because the spikelets are paired and has shown that the dropping of rows in the ear is due to one or more rows of paired spikelets having been discontinued and not to the dropping of one row from each of two or more rows of paired spikelets as Collins assumed.

Each of the paired spikelets consists of two florets, the lower floret usually aborting. However, in certain corns such as the sweet variety, Country Gentleman, both florets are often fertile, and Weatherwax (1935) showed that the crowding thus brought about by the extra grains breaks up the linear arrangement of rows and produces ears without distinct rowing.

Certain abnormalities of the ear have been described as, for instance, reversed kernels where the germ faces in a different direction from the normal. This condition was noted by Kellerman in 1891.

Studies of the early morphological development of the ear have been made by Fujita (1939) and Bonnett (1940). Fujita showed that in ears having odd number of pairs of rows (10, 14, 18, etc.) the rows tend to be twisted but that with even pairs of rows (8, 12, 16, 20) they tend to be straight. He is also the first to publish illustrations of thin cross-sections of the young developing ear. Bonnett (1940), by dissecting out young inflorescences in various stages, has presented a clear picture of the development of the superficial features of the ear. He has shown that the spikelet-forming branch initials divide into two unequal parts to form spikelet initials which then divide into two unequal parts to form the flower initials. In the ear the flower developing from the larger (upper) flower initial becomes fertile and the smaller (lower) one aborts in those corns in which there is a single fertile flower per spikelet. Subtending each spikelet-forming branch is a ridge which increases in size and forms the cupule in which the spikelets are borne.

Cutler (1946), one of the most recent authors who has studied the maize ear, stresses the point that the general opinion still persists that the origin of the ear must have been something unique and nearly miraculous. He discusses a number of grasses which do not ordinarily bear ear-like structures but on which they are found occasionally. These he shows are the result of fasciation. He does not imply, however, that these grasses are directly concerned in the evolution of maize but merely uses them as examples to show that the development of the ear and tassel may not be as unique a phenomenon as it is usually thought to be. He also describes an interesting arrangement of spikelets and alveoli found in a South American corn in which the alveoli, instead of having the linear arrangement as in other corns, are arranged like bricks in a wall.

Cutler also stresses the need for a careful comparative study of the ear. If a number of ear characters can be found which could be used in the classification of corns, he points out, it would be possible to include in the studies of the origin and distribution of the various races of maize the vast number of prehistoric ears which the archaeologists have collected.

#### METHODS

Any one who has handled mature corn cobs is aware that they may be very hard and tough and that some are almost impossible to break with the bare hand. Because of this excessive hardness it is necessary to devise a method whereby serial series of sections could be obtained thin enough for microscopic study. A modified celloidin method was developed which worked well with all materials used including prehistoric cobs from archaeological sites.

Ears of the desired varieties were shelled and the cob boiled for several hours in water to which had been added a few cc. of an aerosol solution. The solution is very effective in small quantities and was prepared by dissolving a small amount of American Cyanamid & Chemical Corporation "Aerosol OT," 100 per cent, in methyl alcohol and then adding an equal amount of water. The only precaution to be taken in its preparation is that the solid aerosol be dissolved in the alcohol before the water is added. The addition of a few cc. of this stock solution has the effect of lowering the surface tension of the water sufficiently so that dry cobs would wet immediately and sink, consequently reducing the time required for boiling. This thorough wetting of the cob made it possible to remove certain gummy substances which were not removed when they were boiled in water without the aerosol. After boiling, the cobs were washed in running tap water for several minutes and then cut into sections small enough to clamp into the microtome. Even after boiling it was often necessary to use a hammer and a heavy scalpel to cut the cob into sections. Small cobs were cut crosswise into sections approximately one inch long. Larger cobs were cut longitudinally into quarters or halves depending upon their size.

The material was dehydrated by running it through an alcohol series using 30, 70, 95 and 100 per cent strengths. Material was left in each of the various grades of alcohol 24 hours.

Two celloidin methods were tried, the regular one as outlined by Chamberlain (1932) and the Rapid Embedding Low Viscosity Nitrocellulose Method outlined by Koneff and Lyons (1937). Results obtained by either method were satisfactory, but since the latter is more simple and more rapid it was the one used in this investigation.

After dehydration the material was placed in a solution of equal parts of absolute alcohol and ethyl ether, where it was allowed to remain for one hour. It was then placed in small wide-mouthed bottles and a 10 per cent nitrocellulose solution added, after which the corks were securely wired in place and the bottles placed in a paraffin oven for 24 hours. The pressure built up in the bottles facilitates the infiltration of the nitrocellulose solution into the plant material. After removal from the oven the bottles were allowed to cool before opening. The 10 per cent nitrocellulose solution was then poured off and a 25 per cent solution added, after which the corks were secured and the bottles again placed in the oven. The following day a 50 per cent solution was added and the bottles placed in the paraffin oven as before. Upon cooling they were opened, and the material picked up by means of forceps and quickly plunged into chloroform where it was allowed to remain for 24 hours in order to harden the nitrocellulose. The chloroform was changed once during the 24 hours. Later the hardened blocks containing the embedded material were placed in 80 per cent alcohol where they were allowed to remain until sectioned.

All sectioning was done with a Bausch & Lomb sliding microtome. Because of the differences in the density of the tissues of the cob, microtome blades must be exceptionally sharp. All the blades used in this investigation were either sent to the factory or to a professional instrument grinder for sharpening. The number of sections obtained before resharpening of the blade is necessary varies with the hardness of the cob but usually from three to five cobs may be cut before the blade needs to be resharpened.

The nitrocellulose blocks were removed from the alcohol and excess embedding material was removed after which the blocks were clamped directly into the microtome. During the cutting the knife and block containing the embedded cob were kept flooded with 80 per cent alcohol, this being accomplished by "painting" them with a small camel's-hair brush dipped into the alcohol. Sections were prevented from curling by holding the brush on the surface of the block as they were cut. The individual sections were removed with the same brush and placed in the alcohol.

All staining was done with safranin and light green. Before staining, the nitrocellulose was removed from the plant material by placing the sections in equal parts of ether and absolute alcohol in small culture dishes for approximately 10 minutes. They were then washed in 95 per cent alcohol and placed in a dish containing water to which was added a few drops of a standard safranin solution (1. g. safranin, 100 cc. 50 per cent alcohol). Sufficient safranin was added to give the water a light pink color. The material was left in this stain over night

and the following day the sections taken out and the excess stain removed by placing them in 95 per cent alcohol to which had been added two drops of concentrated HCl per 100 cc. alcohol. The sections were destained until only a slight pink color remained in the parenchyma cells after which they were washed in 95 per cent alcohol and counterstained in light green dissolved in clove oil. Enough stain from the stock solution (1 g. light green, 100 cc. clove oil) was added to pure clove oil so that the sections would be sufficiently stained in one minute. They were then washed quickly in absolute alcohol, cleared in xylol, and mounted in Canada balsam.

Cobs from archaeological sites were handled in the same way except that after being boiled they were placed in hydrofluoric acid for a few days to dissolve any sand that might be present. The hydrofluoric acid was diluted to 50 per cent with water and placed in paraffin containers. The cobs were then placed in the acid and the containers placed under a hood. On removal from the acid the cobs were thoroughly washed in tap water, dehydrated, and handled like the other material.

#### TOPOGRAPHY OF THE MAIZE COB

It is difficult to interpret the morphology of an ordinary corn cob because its main features are obscured by the papery edges of the floral parts. Even under a dissecting microscope the cob (pl. 37, fig. 1) appears to be a mass of crowded wrinkled chaff of no apparent significance and with the mature cob it is difficult to remove enough of this tissue paper veil without at the same time destroying the underlying structures. Weatherwax (1935) prepared cobs for study by turning them in a lathe and removing all parts down to the hard rachis. Reeves (1946) successfully prepared cobs for study by treating them with sulphuric acid for twelve hours and then washing them in water.

One of the best places to study the surface of the cob is in the corn field. In young growing ears which have been fertilized but are not yet fully mature it is possible to snap off the spikelets with the fingers, leaving the rachis free from the parts that make the mature cob so difficult to study. A morning spent in examining young ears in a large collection will disclose the great variation to be found in the surface features of the cob. Corns possessing the gene (Vg) for vestigial glumes produce ears in which the glumes are so reduced that it is possible to study (pl. 37, fig. 2) the surface features of the rachis without any preparation. The outer glume, while present, is greatly reduced and the lemmas and paleae are almost completely suppressed, thus producing a cob with none of the chaff that makes ordinary cobs so difficult to study.

A typical ear of *Zea Mays* may be said to consist of a more or less elongated cylinder of hardened tissue (the cob) to which are attached pairs of fertile spikelets, usually borne in longitudinal or somewhat spiral rows. If the grains are removed, as well as the chaff and the glumes, to the point where the spikelets are attached to the rachis, it will be seen that the pairs of florets are attached near the lower outer edge of a depression or alveolus that extends into the thickened rachis.

Sturtevant (1899) called this depression a "cupule." Harshberger (1893) wrote, "spikelets . . . placed in a cucullate depression on a fleshy cob." Collins (1919) proposed the word "alicole" to designate "the spikelets, whether staminate or pistillate, that are borne in a single alveolus or at a single point on the rachis, considered as the axil or point of attachment of a reduced branch." Cutler (1946) recently has used *alicole* in a slightly different sense, thus: "each pair of grains arises from a pair of spikelets and comprises with the related parts of the cob the *alicole*." For brevity and clarity it is desirable to have a single word for the structure which has been called the "cucullate depression," "corneous cupule" or "*alicole*" by the different authors. Since *alicole* has been used in different ways it seems best to follow Sturtevant and call the structure a cupule. Throughout this discussion the word "cupule" will be used to designate the depression or alveolus on the cob near the base of which the paired spikelets are attached.

Weatherwax (1935) has shown that the pairs of spikelets on the ear not only maintain a linear relationship but also a lateral one, and he showed that the spikelets of one row were not opposite those of the adjacent row but alternate with them. He also showed that the dropping of rows in the ear is due to one or more rows of paired spikelets having been discontinued and not to the dropping of one row from each of two or more rows of spikelets as Collins (1919) had assumed. Cutler (1946) has described a race of maize from South America in which the cupules are arranged like bricks, each being covered with half a cupule from the right and half a cupule from the left, thus forming an ear with cross-spiralling of the rows. In other words, rows can be traced in a spiral around the ear in two directions. Thus a single grain will be part of a row that spirals to the left and also part of a row that spirals to the right.

Cupules are not unknown in other grasses. They are conspicuous in such genera as *Manisuris*, *Hackelochloa*, and *Tripsacum*. St. Augustine grass (*Stenotaphrum secundatum* (Walt.) O. Kuntze) bears rows of solitary fertile spikelets in cupules on a corky flattened rachis (Cutler, 1946). On close examination it will be seen that the cupules of different varieties of maize vary greatly in shape, in the depth to which they penetrate into the cob and in the development of tissue on the sides above the attachment of the spikelet pairs. In some varieties the cupule is shallow and almost saucer-shaped while in others it is much wider than it is high and penetrates deeply into the rachis. In many of the corn-belt varieties the cupule not only penetrates deeply into the rachis but tends to turn up at an angle. According to Bonnett (1940) the cupule develops from a ridge subtending the spikelet initials and is similar to the subtending leaf initials that appear in the differentiation of the inflorescence of barley, wheat, and oats. The development of the outer edge of the cupule was mentioned by Harshberger (1893) in describing *Zea Mays*: "spikelets paired in alveoli, strongly marginal and cupulate, the margins becoming hard and corneous." Arber (1934) has used the term "rachilla-flaps" for the non-vascularized outgrowths of the reproductive axis of certain grasses and has given as an example the outgrowths of the lip of an oblique cupule in



*Cephalostachyum virgatum* Kurz., an east Indian bamboo. Cutler (1948) has called the outgrowths of the margin of the cupule in maize, "rachis-flaps," and that term will be used in the following discussion. In certain races of maize the rachis-flaps are little developed and form only a slight ridge separating the cupules from the adjacent parts of the cob. In other races the rachis-flaps form very conspicuous outgrowths at the side of the cupule which may be several mm. in length, as, for example, the Mexican variety Cacahuazintle, which has rachis-flaps as much as 5 mm. in length and is flexible due to the presence of only a small amount of sclerenchyma. In varieties with highly sclerenchymatized cobs the rachis-flaps are usually hard and corneous.

Another variation apparent in cross-sections of the cob is the manner of insertion of the spikelet pairs on the cob axis. This is affected to a certain extent by the development of the rachis-flaps as well as by the depth of the cupule. Cross-sections of certain varieties show the spikelets attached directly to a more or less cylindrical rachis, while other cobs, especially United States corn-belt varieties, have the spikelets attached at the bottom of a cup-like depression. A closer inspection will disclose that this difference is due to the fact that in the first case the cupule is very shallow and the rachis-flaps only slightly developed while in most corn-belt varieties the cupule is well developed and extends a considerable distance into the rachis and the rachis-flaps tend to be well developed.

The length of the pedicels of the spikelets varies in different races of maize and has been mentioned by various investigators (Mangelsdorf & Cameron, 1942; Cutler, 1946). In some races of corn such as the northern flints as defined by Brown and Anderson (1947) the spikelets are attached by a broad base to the lower margin of the cupule so that there is no definite line of separation between the glumes and the cob. In certain varieties from South America and from Asia the spikelets are distinctly pedicellate, and the pedicels may be up to 2 mm. in length.

#### INTERNAL ANATOMY

Just as the cob varies in its surface features, so it also varies in its internal features. Cross-sections show great variation in the proportion of sclerenchyma and pith. Four distinct zones may be differentiated in the cross-section of an average corn-belt cob as follows: (1) Immediately inside of the epidermis there is a more or less solid ring of thick-walled lignified cells inside of which there is, (2) a cylinder of pith composed of large thin-walled parenchyma cells. Lying embedded in these two zones is (3) the vascular network which, at least in certain races of maize, consists of two rather distinct tubes of vascular bundles, one inside of the other (Reeves, 1946; Laubengayer, 1946). Their location relative to the other zones will be discussed more fully later. (4) The fourth zone consists of branched parenchyma cells which surround the vascular bundles as they leave the inner vascular tube and extend out into the spikelets.

*Sclerenchyma.*—

The texture of the cob, whether fibrous and flexible or lignified, hard and stiff, depends to a large extent upon the relative amount of sclerenchyma contained. Most North American corn-belt varieties have a hard tough cob. The cross-sections of such types show a well-defined sclerenchyma zone forming a nearly solid tube between the epidermis and the pith. It is within this mass of thick-walled cells that the cupules are located and in such varieties the rachis-flaps, as well as the outer glumes of the spikelets, tend to be heavily sclerenchymatized. There are other races of maize which tend to have rather soft cobs, as, for example,

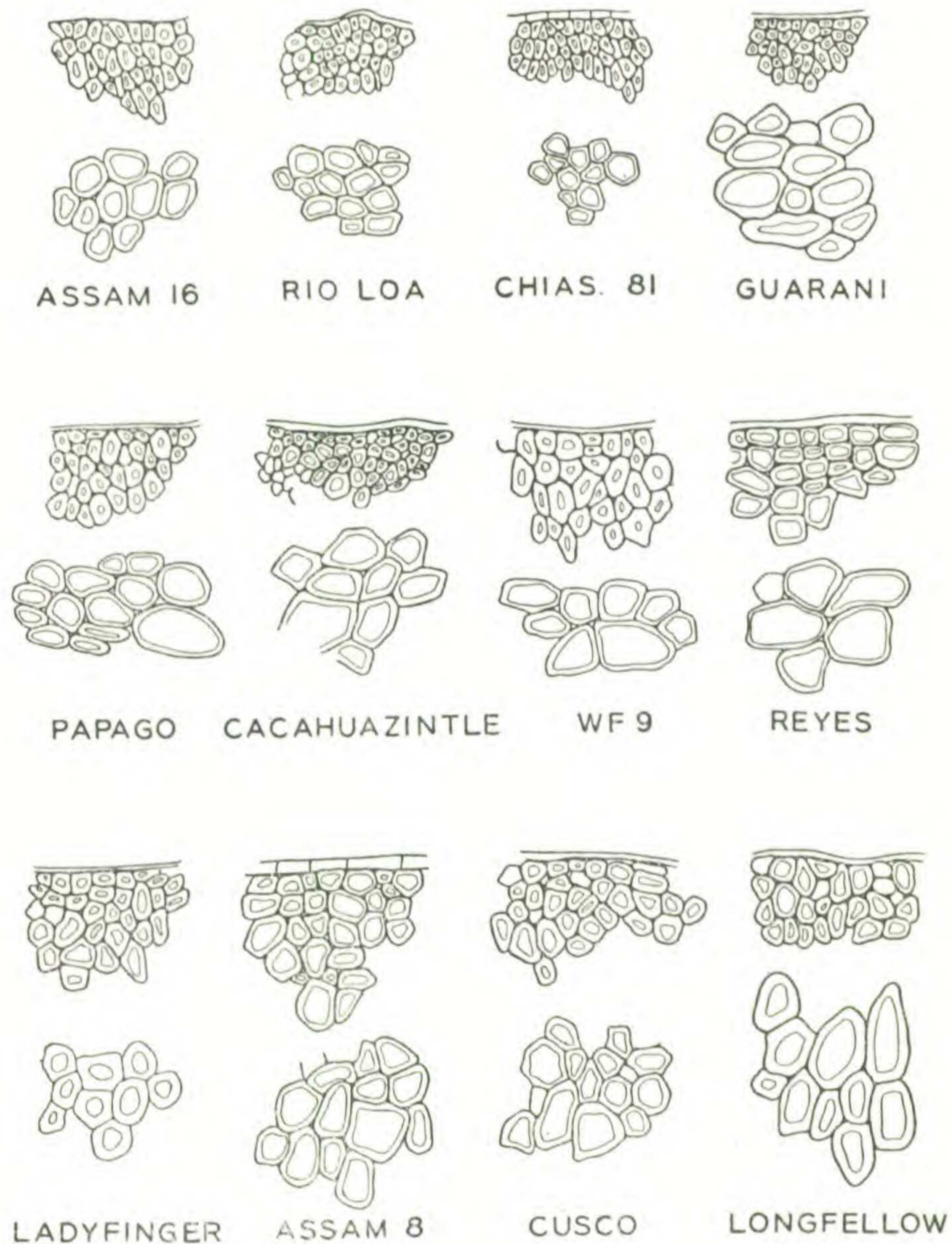


Fig. 1. Camera-lucida drawing of sclerenchyma. In each case upper drawing shows cells from immediately under the epidermis, lower drawing shows them half way between the epidermis and the pith.

certain South American varieties which are so delicately constructed that one can push the grains into the cob with the finger (Mangelsdorf & Cameron, 1942). In such cobs the sclerenchyma is present only as a relatively thin layer surrounding the cupules, and the rachis-flaps tend to be sclerenchymatized only on their inner face, while the glumes (Cutler, 1946) may even be delicate membranes.

The sclerenchyma tissue itself varies considerably in different corns. The cells may be relatively small and densely packed or larger and rather loosely packed. The distinction between sclerenchyma cells and pith cells, while abrupt and distinct in most corns, may show a rather wide area of transitional tissue where the cells become progressively larger and less thick-walled as they approach the central pith area.

Within any one cob the shape of the sclerenchyma cells varies greatly depending on their location. Both sclereids and fibers are found. According to Eames and MacDaniels (1947), the classification of sclerenchyma into sclereids and fibers has no morphological significance and is only used for convenience in description. In the sclerenchyma of the cob there is every gradation from typical fiber cells many times as long as wide to isodiametric sclereids. They may be smooth in outline or variously lobed. One unusual type of cell present in the cob is the branched parenchyma cell discussed in detail below. In some areas these cells become thick-walled and lignified and can thus be termed "astrosclereids." The size of the sclerenchyma cells and the amount of thickening of the cell wall vary in different races of corn as shown in fig. 1. The pits are small and the pit canals are usually unbranched. This character, as shown by Eames and MacDaniels (1947), is of little value in classification, being dependent to a large extent upon the thickness of the cell wall.

#### *Pith.*—

Nearly all corn cobs possess a central cylinder of pith. Prehistoric cobs often appear to be hollow, due to the decomposition of the soft parenchymatous tissue that originally filled the center of the cob. In general, the pith is rather uniform in the different races of maize, the cells being nearly isodiametric and thin-walled. However, the cell size varies in different corns, some varieties having more and larger intercellular spaces than others. As has already been mentioned, there is sometimes a rather wide area of transitional cells between the thick-walled sclerenchyma and the thin-walled parenchyma. There is also an area of transitional cells between the regular parenchyma pith cells and the highly branched parenchyma cells which form the sheath around the vascular bundles as they enter the spikelets. In the cobs of certain corns there may be a ring of cells in the pith which are colored and slightly thicker-walled than the surrounding pith cells. These are the so-called "pith-rings" of the corn-breeders. Pith-rings are commonly found in North American corns and have also been found in early prehistoric corn from Arica, Chile.

*Vascular System.*—

It has been difficult to obtain a complete understanding of the vascular system of the corn plant because of the difficulty in making preparations of certain portions, especially of the node and ear. Evans (1928), in discussing the vascularization of the node of *Zea Mays*, wrote:

"Imbedding of tissue from the node and then sectioning has been found inadequate in the study of the node, since the branching of the vascular bundle is so intricate and the branches so tortuous as to defy solution even in the best prepared serial sections."

He developed a method of staining the vascular bundles in the stalk and then subjecting the sections to retting action by bacteria. After removal of the decomposed parenchymatous cells he was able to study the vascular network of the node of the corn stalk. He concluded that single vascular bundles seldom pass through more than two or three nodes without branching and further that "so complicated does this branching become that it is impossible of tracing even when no other tissues are present to complicate the situation."

Reeves (1946), in studying the vascular system of the mature ear, also used a retting process involving cellulose-digesting microorganisms. He was able to separate the vascular system from the other parts of the cob. Laubengayer (1946), using much the same technique, studied the vascularization of the cob of a northern flint. He showed that the vascular arrangement of the shank was essentially that of the main stem. However, in the transitional region from shank to ear the bundle system reorganized itself into two distinct systems, one inside of the other. The inner vascular system, with its larger bundles, supplies the spikelets, while the outer tube of smaller and more-branched bundles supplies the other parts of the cob. This arrangement of the bundles as shown by Laubengayer differs from that described by Weatherwax (1935), who says that the vascular bundles of the cob are distributed in approximately the same manner as those of the stem except for a tendency toward peripheral arrangement. Laubengayer's interpretation would seem to hold true for the majority of the cobs examined in this investigation. There are, however, varieties in which the vascular bundles are more or less evenly distributed throughout the pith of the cob, as, for example, in the inbred line Illinois R4, and in such cases it would appear that the inner vascular system would then form more of a cylinder of vascular bundles rather than the hollow tube of bundles as demonstrated by Laubengayer for the northern flints. That these two vascular systems are not entirely free from one another has been shown by Cutler (verbal communication). He was able to find small branches which connect the two systems at the nodes and he also found that a bundle that is originally a member of the inner system may later become a member of the outer system.

The location of the two fibrovascular systems relative to that of the sclerenchyma tissue varies greatly. In general, in cobs with large amounts of sclerenchyma, as, for example, those of the North American corn-belt varieties, the outer vascular system lies embedded within the sclerotic tissue. The inner cylinder, the

one supplying the spikelets, tends to lie along the line separating the sclerenchyma from the pith. Some bundles, however, may be located in the pith a slight distance from the sclerenchyma layer, in which case there may be a lobe of sclerenchyma tissue extending into the pith and surrounding the bundles with a sheath of sclerenchyma tissue a few cells thick. Since all the bundles are surrounded by a sclerenchymatous bundle-sheath it would appear in some cases that this lobe of sclerenchyma tissue does not actually surround the bundle itself because the layer of sclerenchyma cells around the bundle is no thicker than that found in the regular bundle-sheath. In some varieties such as inbred line Illinois R4, a considerable number of vascular bundles will be found distributed throughout the pith zone. In most corns some vascular bundles of the inner system will be found embedded within the sclerenchyma.

In races of corn which have cobs less sclerenchymatized than the rather extreme corn-belt varieties, the outer vascular tube tends to occupy the parenchymatous areas lying between the masses of sclerotic cells.

The vascular bundle of the corn plant has long been used in elementary classes as a typical example of a vascular strand, and since so many writers (Hayward, 1939, Holman & Robbins, 1939) have described the cells of which it is composed it need not be discussed here. No essential difference is found in the structure of the bundles of the two systems except that of size.

#### *Branched Parenchyma.*—

One of the most unusual types of tissue found in the maize cob is the branched parenchyma cells which are located between the sclerotic tissue and the vascular strands near the base of the spikelet and extending some distance into it. According to Eames (personal communication) the presence of these cells in corn is surprising, as is also their location. The inner edge of this zone lies near the inner vascular system, and cells transitional between pith parenchyma and the branched parenchyma may be found. The branched parenchyma cells tend to be considerably smaller than the pith parenchyma cells and are usually somewhat elongate with numerous branches, although they may be almost stellate in shape. The tips of the branches often are enlarged and may be nearly twice the diameter of the rest of the branch. The cell walls of these cells are usually thin but near the sclerotic zone some of the cells may be lignified, and in such cases they could be classified as "astrosclereids."

#### DISCUSSION

Of the anatomical and histological characters of the cob which are capable of exact measurement three characters vary greatly between the different varieties, namely, (1) The rachis-flaps, (2) the length and shape of the pedicels, and (3) the amount and distribution of the sclerenchyma.

(1) *Rachis-flaps.*—One of the most conspicuous differences in the cross-sections of the various varieties of maize is found in the rachis-flaps which vary in

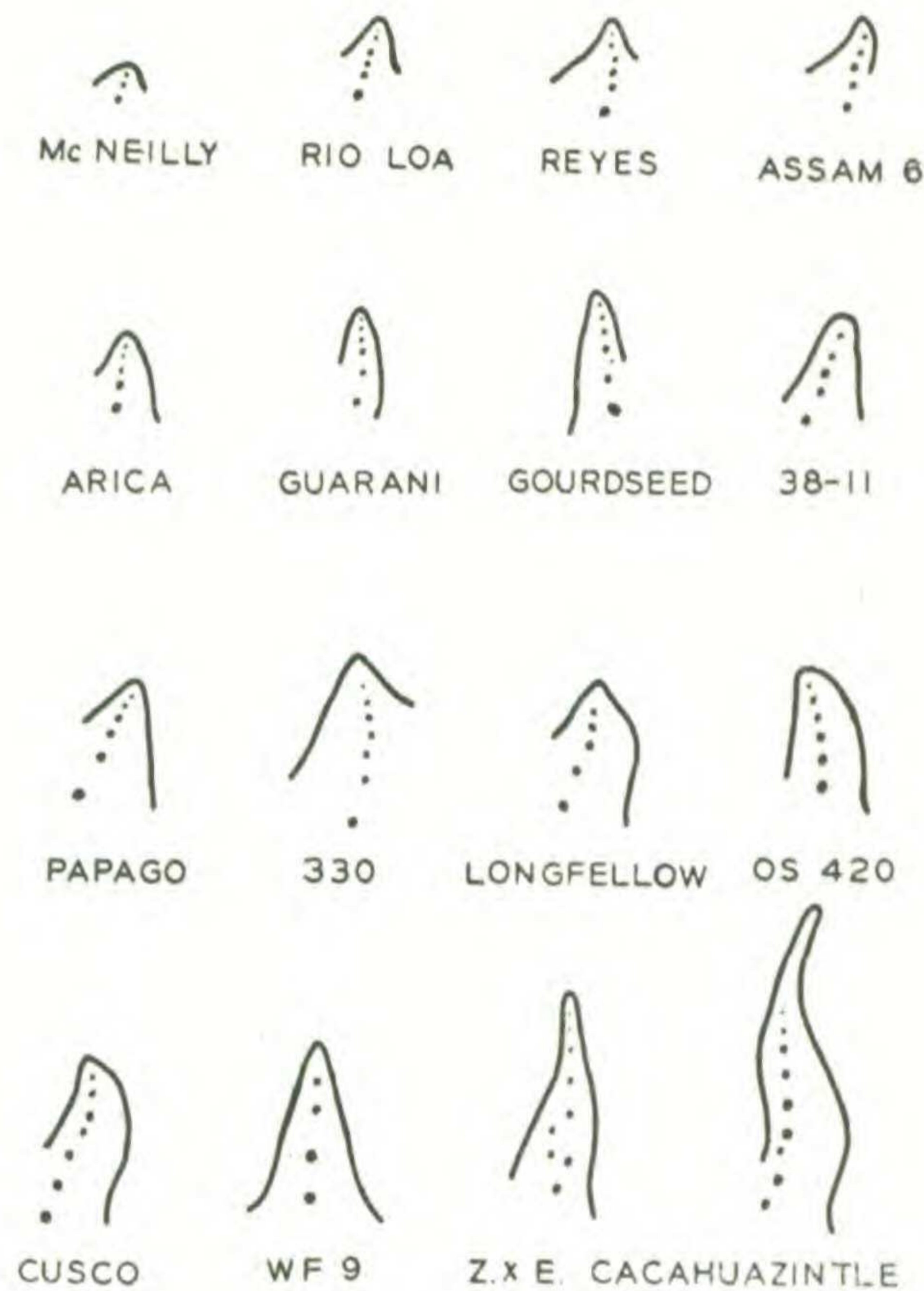


Fig. 2. Outline drawings of cross-sections of rachis-flaps made at the point of greatest development. Black dots are vascular bundles.

both size and shape. As defined in this paper, rachis-flaps are the outgrowths from the side of the cupule. They vary from a slight ridge separating the cupule from the adjacent parts of the cob (as, for example, in Ladyfinger popcorn) to the extremely long flexible appendages found in the Mexican variety, Cacahuazintle. To obtain an exact measure of the rachis-flaps, their outline was traced on paper from prepared cross-sections by means of a Bausch & Lomb microslide projector. The extent of variation of this character is shown in fig. 2. It is evident that Ladyfinger, Rio Loa, Reyes, Bolivia and Assam #6 and the prehistoric Arica varieties have the least developed rachis-flaps and that the most strongly developed ones are found in North American and Mexican varieties—inbred line WF 9, *Zea-Euchlaena* back-crossed to *Zea*, and Cacahuazintle.

From the varieties so far studied it would appear that the presence of well-developed rachis-flaps is due to *Tripsacum* contamination. The evidence for this, while not conclusive, is suggestive. Except for Cacahuazintle, the greatest development of rachis-flaps is found in the *Zea-Euchlaena* cross and all other varieties possessing well-developed rachis-flaps also show other tripsacoid characters. Cacahuazintle is a high-altitude Mexican variety. From the criteria advanced by Mangelsdorf and Reeves (1939) Cacahuazintle would in general be non-tripsacoid.

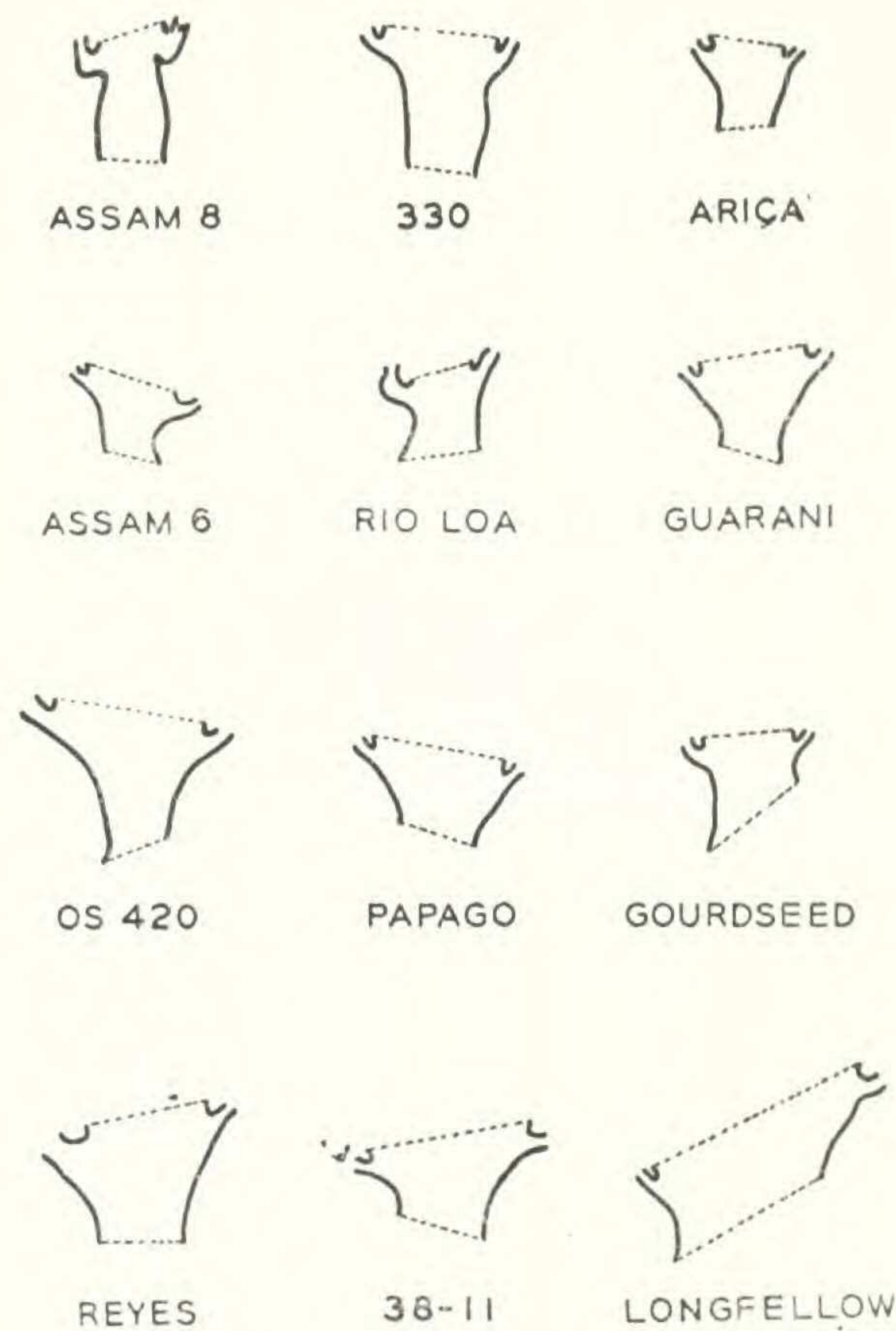


Fig. 3. Camera-lucida drawings of longitudinal sections of pedicels and glume bases.

The development of the excessively long rachis-flaps might be explained as due to an extreme recombination of characters derived from *Zea* and from *Tripsacum*.

(2) *Pedicel Length*.—As has already been mentioned by several authors (Mangelsdorf, 1945; Cutler, 1946) the length of the pedicel varies in different varieties of maize. They have described the long pedicels found in certain South American varieties. The pedicel not only varies in length but also in width and in shape; some of the South American and Oriental varieties have long and thin pedicels while other varieties may have equally long but relatively much thicker ones. In order to obtain a measure of this character slides were projected as before and the outline of the pedicel traced on paper. Since length and thickness are both important, the length of the pedicel was divided by the width in order to arrive at a significant measure of the relative length and thickness of the structure. Results are shown in fig. 3. The longest pedicel found in any variety studied was in Assam #8 (see pl. 38, fig. 6). In this variety the pedicels are rather thin and may be as much as 2 mm. in length, equal to any reported by Cutler for Andean corn. The fact that the pedicels are borne in a shallow cupule makes their length more noticeable than if the cupule were deep. In the commercial four-way hybrid, Pioneer 330, which has rather extremely long pedicels for North American corn,

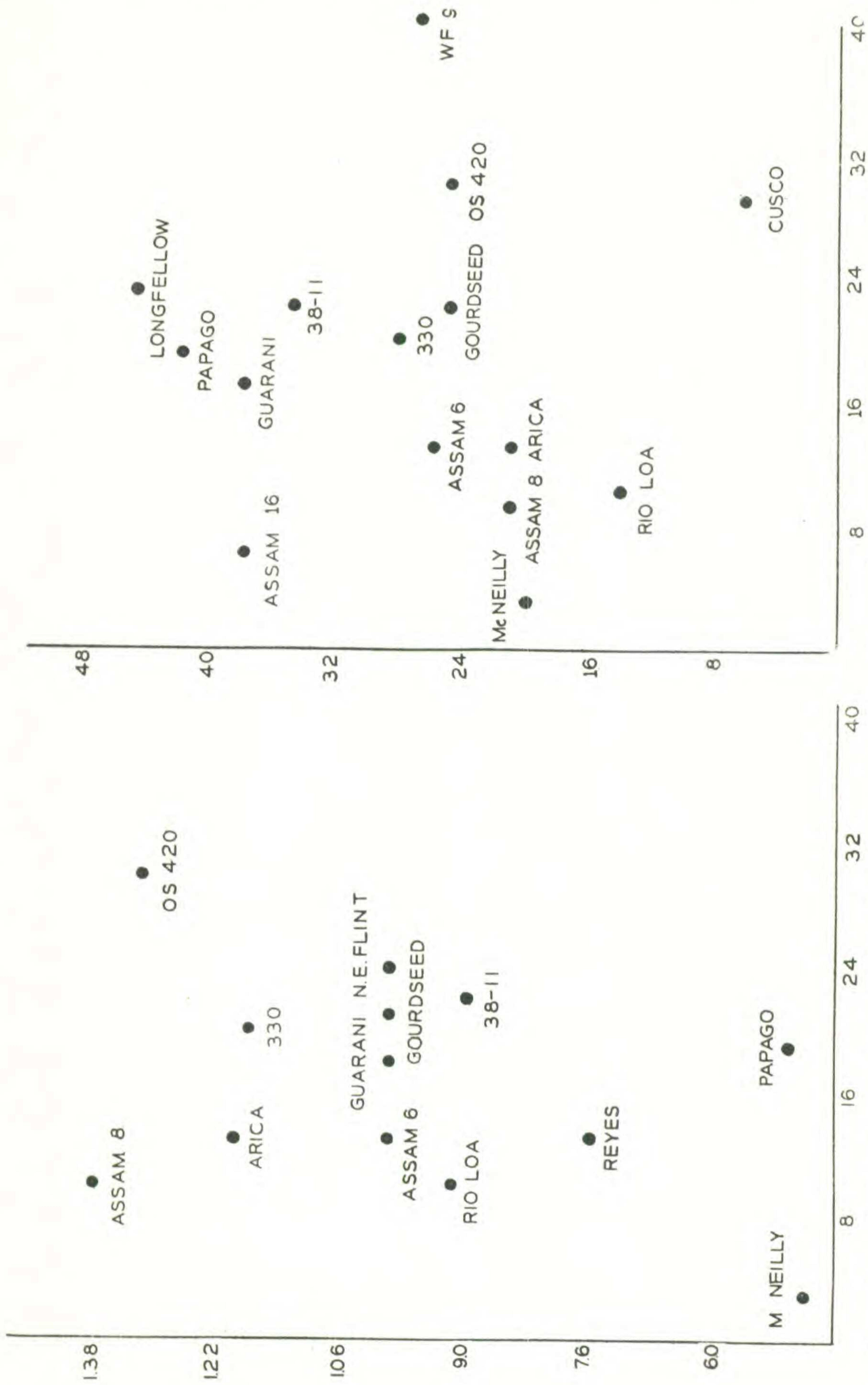


Diagram I. Scatter diagram showing relationship between pedicel length and length of rachis-flaps: horizontal axis, length of rachis-flap in mm.; vertical axis, pedicel length in per cent of pedicel width. Note that "McNeilly" in diagram is referred to as "Ladyfinger" in the text.

Diagram II. Scatter diagram showing relationship between rachis-flap length and per cent of sclerenchymatization of the cob: horizontal axis, rachis-flap length in mm.; vertical axis, per cent of sclerenchymatization of cob.



they are less conspicuous than in the South American and Oriental varieties since they are set in a deep cupule. The pedicels in Pioneer 330 are also broader in relation to their length than those in Assam #8. Among those varieties having short and thick pedicels are Gourdseed, Longfellow and New England Flint. According to Cutler (1946) one of the most obvious effects of tripsacoid influence is the shortening of the pedicels and the sinking of the whole spikelet into the cupules.

(3) *Sclerenchyma*.—Stained sections show great intervarietal differences in the amount of sclerenchyma. This is noticeable from stained slides even without the use of a microscope. Since the distribution of the sclerenchyma, as well as the amount, varies from variety to variety, it was necessary to measure the differences in the same relative place on all the cobs. After some preliminary trials it was decided to measure the thickness of the sclerenchyma at the point where the cupule extended the greatest distance into the cob. Slides of cross-sections were projected and the outline of the cupule traced showing the extent of the sclerenchyma. This was then divided by the radius of the cob at that point. This was called the degree of sclerenchymatization of the cob. It varies in the different varieties from 5 to nearly 50 per cent of the cob at the point measured. Since this character varies considerably within a single cob all sections used for comparative study were from near the center of the cob.

The least sclerenchymatized variety studied is Chiapas #81 (Rockefeller Foundation collection number), a flexible-cobbed variety. Here the sclerenchyma forms only a thin layer surrounding the cupule. Other varieties possessing small amounts of sclerenchyma are Rio Loa, Cusco, Assam #8, Arica and Ladyfinger popcorn. Among the most highly sclerenchymatized varieties are Longfellow, Papago, Guarani and inbred line 38-11. According to Mangelsdorf and Cameron (1942) *Tripsacum* contamination tends to impart a coriaceous quality to all parts of the cob. Of the highly sclerenchymatized varieties studied all possess other characters which would indicate that the variety is highly tripsacoid.

#### SUMMARY

1. The anatomy of the female inflorescence of *Zea Mays* (the corn cob) is of importance in corn-breeding, in studies of archaeological maize, and in considering the phylogeny of maize and its relatives.

2. Thin sections of mature corn cobs, both of recent and archaeological material, were prepared by a modified celloidin technique which is described in detail.

3. The topography of the mature cob is described. The main features of the cob surface are: (a) the paired spikelets, (b) the associated cupule (or alveolus) and (c) the outgrowths from the side of the cupule, the rachis-flaps. The pedicels of the spikelets vary greatly in different varieties of maize, both in length and in shape. Certain varieties have pedicels as much as 2 mm. in length. Cupules vary from shallow depressions on the rachis, to deep cavities

penetrating a considerable distance into the cob. The rachis-flaps vary from slight ridges separating the cupule from the adjacent parts of the cob to long flexible appendages as much as 5 mm. long.

4. In cross-sections of mature cobs four distinct types of tissue are found, exclusive of the epidermis. They are: (a) A sclerenchyma zone, (b) a cylinder of pith, (c) the highly branched vascular system which consists (at least in certain varieties) of two rather distinct cylinders of vascular elements one inside of the other. The inner system supplies the spikelets and the outer system supplies the other parts of the cob, and (d) branched parenchyma cells which surround the vascular strands as they leave the inner vascular cylinder.

Great variation exists in the amount and location of the sclerenchyma in the cob, and varieties with "soft" cobs are shown to contain smaller amounts of sclerenchyma than "hard" cobs. The location of the inner vascular system varies but tends to occupy the area between the pith and the sclerenchyma. The outer vascular system is embedded within the sclerenchyma in highly sclerenchymatized varieties and the area between the masses of sclerenchyma in varieties which are only slightly sclerenchymatized. Nearly all cobs possess a pith center. The size of the parenchyma cells and the number of intercellular spaces vary in different varieties.

5. Three characters were found in the cob which varied greatly between the different varieties of maize, and methods were devised to measure these differences. *Rachis-flaps*: The least-developed rachis-flaps are found in Ladyfinger popcorn, Rio Loa, Reyes, Assam #6 and the prehistoric Arica, Chile, corn. The greatest development of rachis-flaps are in Mexican and North American varieties, Gourdseed, inbred line 38-11, Papago, Longfellow, OS-420, WF-9 and Cacahuazintle. *Pedicel length*: Pedicels vary from short and thick to long and thin. Assam #8, Pioneer 330, Arica, Assam #6 and Rio Loa have relatively longer and thinner pedicels than the varieties, Longfellow, inbred line 38-11, Reyes, and Gourdseed. *Sclerenchyma*: The amount of sclerenchyma in the different varieties of corn varied from 5 to 46 per cent of the radius of the cob at the point measured. The least sclerenchymatized varieties were Cusco, Rio Loa, Assam #8, Arica and Ladyfinger popcorn. The most highly sclerenchymatized varieties were Longfellow, Papago, Assam #16, Guarani and inbred line 38-11.

#### SOURCE OF MAIZE VARIETIES STUDIED

- ARICA—Junius Bird, Am. Mus. of Nat. Hist.—from excavations at Arica, Chile.  
 ASSAM #6—Naga Hill Tribes, Assam, India.  
 ASSAM #8—Naga Hill Tribes, Assam, India.  
 ASSAM #16—Naga Hill Tribes, Assam, India.  
 CACAHUAZINTLE—Rockefeller Foundation Collection, Mexico, D. F.  
 CHIAPAS #81—Rockefeller Foundation Collection, Mexico, D.F.  
 CUSCO—Lehman Collection, Cusco, Peru.  
 GOURDSEED—Grapevine, Texas.  
 ILLINOIS R4—Pioneer Hi-Bred Corn Co., Johnston, Iowa.

LADYFINGER—McNeilly Seed Co.

MCNEILLY—see LADYFINGER.

PAPAGO—L. W. Lenz, Papago Indian Reservation, Sells, Arizona.

PIONEER 330—Pioneer Hi-Bred Corn Co., Johnston, Iowa.

REYES—H. C. Cutler, Reyes, Bolivia.

RIO LOA—Carl and Jonathan Sauer, Chiu Chiu, Chile.

VESTIGIAL GLUME (Vg)—DeKalb Hybrid Seed Corn Co., DeKalb, Ill.

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## EXPLANATION OF PLATE

## PLATE 37

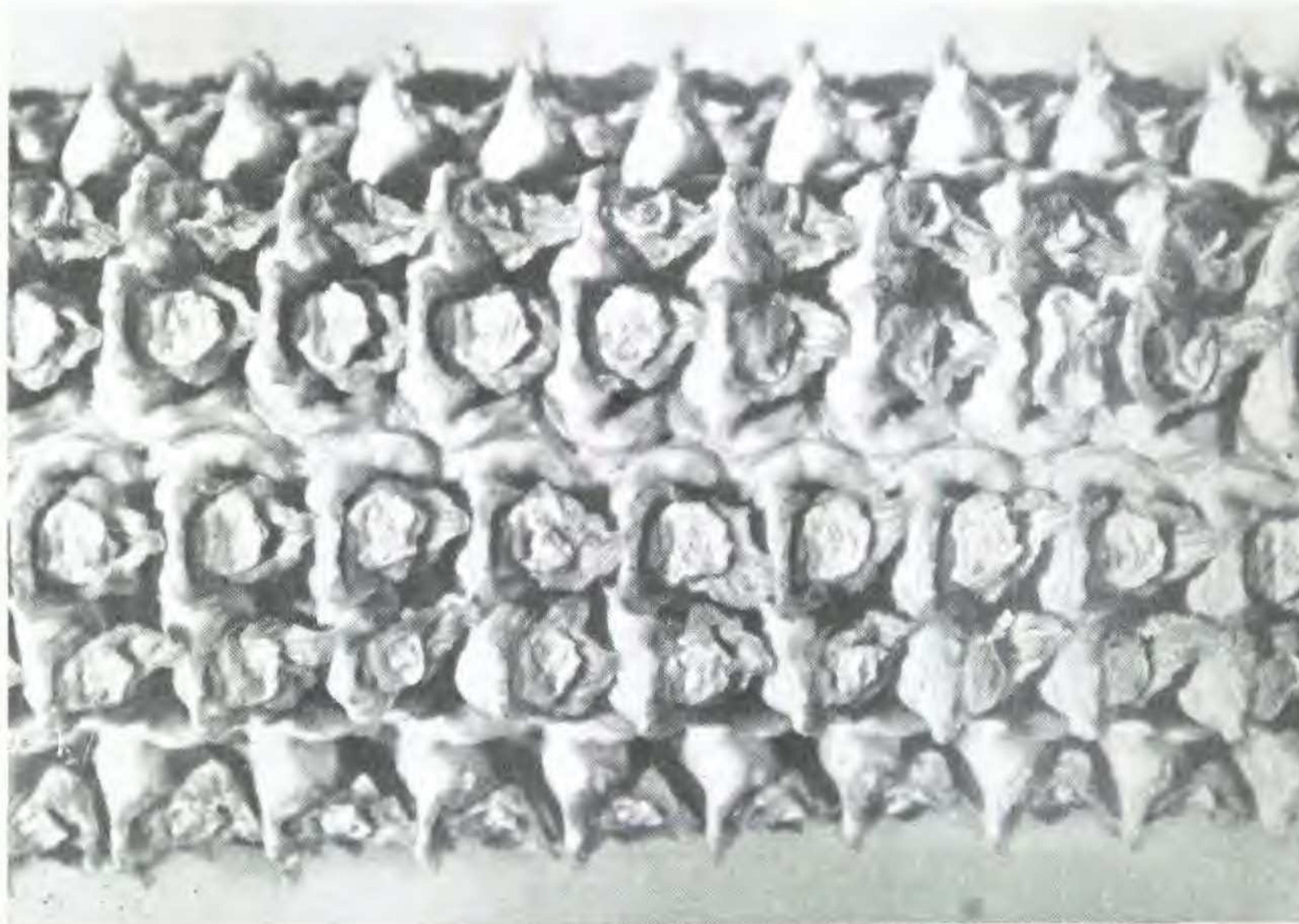
Fig. 1. Surface view of an ordinary cob showing remaining parts of spikelets. Scarios edges of floral parts conceal nearly all of the surface detail of the rachis. The white broken ends of the pedicels can be seen in a few places.

Fig. 2. Surface view of a cob from plant homozygous for vestigial glume (Vg). Reduction of glumes and lemmas reveals the following surface details: (a) paired spikelets, (b) rachis-flaps clearly seen at center of photograph, (c) alternate arrangement of pairs of spikelets on adjacent pairs of rows, (d) cupule extending into rachis behind the broken ends of the pedicel.

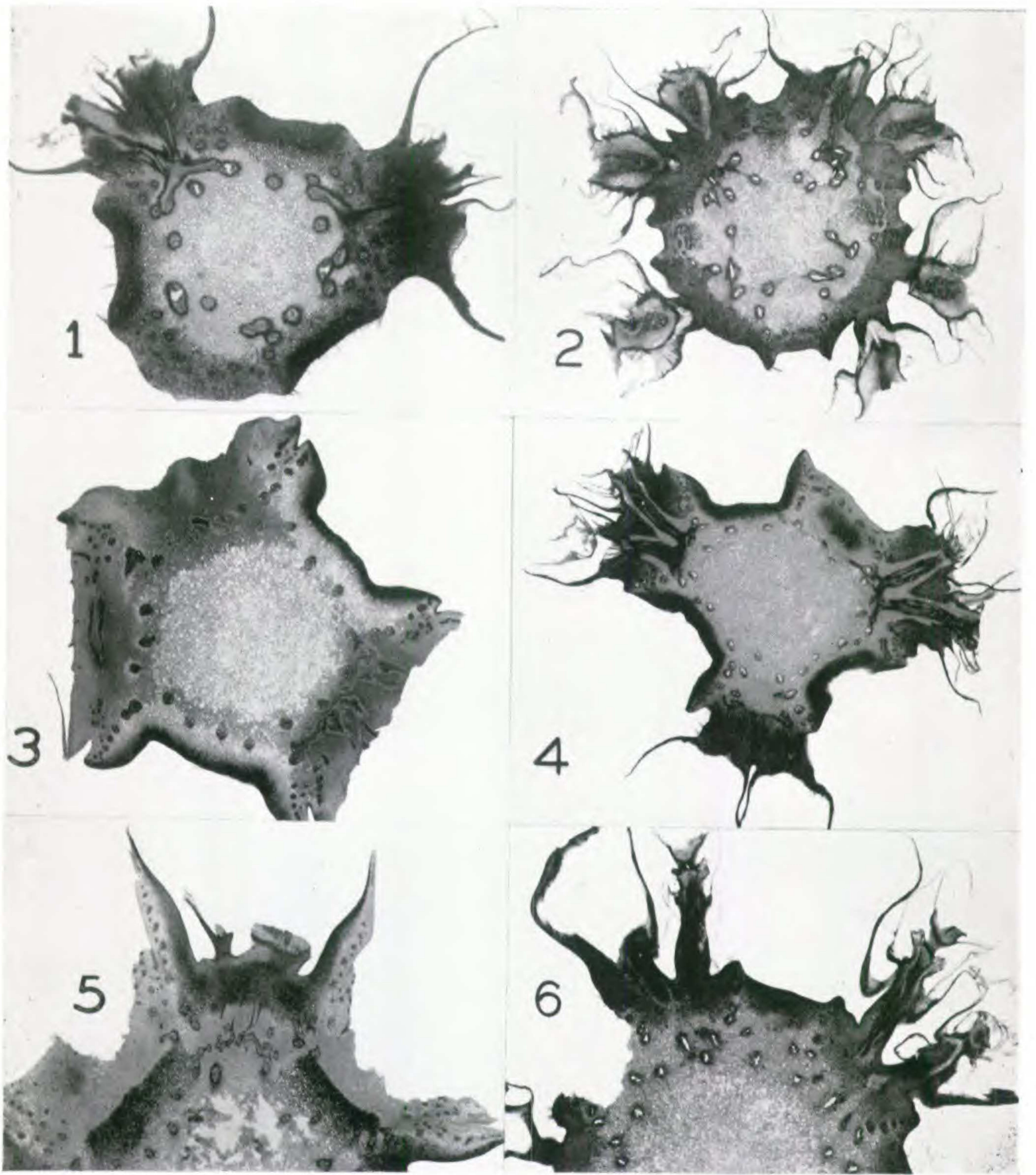


1

LENZ—HISTOLOGY OF THE CORN COB



2



LENZ—HISTOLOGY OF THE CORN COB

EXPLANATION OF PLATE

PLATE 38

Photomicrographs of cross-sections of corn cobs: (1) Ladyfinger, (2) Reyes, (3) Chiapas #81 (flexible cob), (4) Arica, Chile (prehistoric), (5) Cacahuazintle, (6) Assam, India, #8.

## EXPLANATION OF PLATE

## PLATE 39

Camera-lucida drawings of a serial series of cross-sections of the cob of a United States commercial "hybrid" variety (Pioneer 330). Solid black, dense sclerenchyma; stippled, ordinary sclerenchyma; white, parenchyma.

Fig. 1. Showing pair of spikelets set deep in the rachis.

Fig. 2. Beginning of next cupule above and between the spikelets shown in fig. 1; first seen as an area of less dense sclerenchyma inside the center of dense sclerenchyma, later an opening appears in this mass of sclerenchyma.

Fig. 3. Opening which first appears in fig. 2 increases in size outward until it reaches the surface and opens out to form the cupule.

Fig. 4. Cupule with pedicel of spikelet pair rising out of it.

Fig. 5. Cupule with longitudinal section of pair of spikelets.

Fig. 6. Areas of dense sclerenchyma at either side of spikelets show the beginning of the cupules which will appear immediately above the spikelets shown in fig. 1.